

## A SUMMARY OF OUR KNOWLEDGE OF ENDOCRINE MECHANISMS IN CRUSTACEANS\*

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Definite demonstration of the presence of an endocrine mechanism in invertebrates is of quite recent origin, extending back not more than about fifteen years. Biologists have, nevertheless, suspected for several decades that such mechanisms existed among these lower animals. When one considered the fact that almost every basic physiological mechanism found in the Vertebrata appeared to have its counterpart somewhere among the Invertebrata it was not unreasonable to presume that hormonal coordinatory mechanisms were operative there just as in the Vertebrata. Many problems in invertebrate physiology which remained quite refractory to solution solely in terms of nervous coordination recently have yielded much ground in terms of endocrine coordination. A number of general reviews of the subject have been prepared, namely Koller, 1929, Hanström, 1937a, von der Wense, 1937, Koller, 1938, Lelu, 1938, Hanström, 1939, and others are in preparation.

The first invertebrate group to provide unequivocal evidence for the possession of an endocrine mechanism was the Crustacea (Perkins, 1928). And today we probably have as large a body of information concerning crustacean endocrine physiology as we have for any other invertebrate group, with the possible exception of the Insecta. Even in these best known groups, however, our knowledge is still very rudimentary and sketchy. One who is primarily familiar with the relatively refined and detailed state of the field of mammalian endocrinology must look with patience and understanding upon this new field which is only now concerned with the rough determination of hormonally controlled processes, and the sources of the hormones involved. Furthermore, the investigation of invertebrate endocrinology is rendered somewhat difficult by

the small size of most of the animals which must be investigated and also due to the general phylogenetic tendency toward decrease in structural differentiation and segregation of organs or parts of organs concerned with specific functions. This last tendency, of course, culminates in the Protozoa whose total structural bases of functions are confined within the limits of a single cell membrane.

It is obviously impossible within the twenty minutes allotted for this report to cover even in outline the whole of our knowledge of invertebrate hormonal mechanisms. The literature includes more than 1000 articles. Nor is there even time to discuss adequately the experimental evidence upon which our knowledge of a single endocrine gland (i. e. the insect corpora allata or crustacean sinus gland) is based. Instead, a middle pathway has been selected in which there will be an attempt to present a brief and summary account of our present picture of crustacean endocrines, their sources, and the functions which have been assigned to them. It is hoped that too much has not been attempted.

**The Eyestalks and the Sinus Glands.—**

For many years it has been known that many higher crustaceans macroscopically blanch or darken their integument to correspond with the shade of the background upon which they come to lie. These color changes are principally the result of migrations of differently colored pigments within the bodies of highly branched integumentary cells called chromatophores. Any pigment which is dispersed into the branches and ultimate twigs of a chromatophore imparts its color to the general appearance of the animal; conversely, any pigment which is concentrated into a minute knot in the chromatophore center has little or no influence upon the gross

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coloration. By appropriate differential migrations of pigments of several colors a number of crustaceans may simulate more or less perfectly the tints of variously colored backgrounds. Many operative techniques involving cutting of nerves and consequent denervation of areas of the body of crustaceans have failed to interfere with the normal responses of the chromatophores concerned. Thus there is evidently no direct nervous control of these cells. Koller (1925, 1927) gave us evidence from blood transfusions that a blood-borne agent was responsible for certain of these chromatophore responses in the shrimp, *Crago*. Shortly thereafter, Perkins (1928) discovered that water extracts of the eyestalks of the shrimp, *Palaemonetes*, possessed the ability to concentrate dispersed red pigment strongly, thus blanching this animal. Extract of no other part of the body appeared to do this. Koller (1928) confirmed the presence in the eyestalks of *Crago* and of *Leander* of such a chromatophorotropic substance which produced strong concentration of their dark pigments. Koller further demonstrated that the eyestalk substance was not species-specific, but that eyestalk extract of *Leander* was effective upon *Crago* chromatophores and *Crago* eyestalk extract was effective upon *Leander* chromatophores.

During the following few years Kropp and Perkins (1933) tested many stalk-eyed crustaceans and discovered that all possessed such a substance in their eyestalks. Therefore this eyestalk substance which effected blanching of dark shrimp was of quite general occurrence.

In 1935 Carlson, and later in the same year Abramowitz, showed us that, contrary to the condition obtaining in shrimp, eyestalk extract of crustaceans produced dispersion of concentrated black pigment in the crabs, *Uca* and *Portunus*.

Meanwhile, Brown (1935b) had been examining in much detail the structure and responses of the chromatophore system of the shrimp, *Palaemonetes*. After learning that this animal possessed four pigmentary types (red, yellow, white and blue), all of which were able to react relatively independently of one another (even in a denervated area) when the animal was placed upon backgrounds, he concluded that the existence of at least four hormones was indicated

to account for this complex behavior. This conclusion was unnecessary, as has been pointed out by Parker (1940), since three hormones, A, B and C, can be seen to yield seven combinations which could conceivably have as many qualitatively different actions on the chromatophore system. As a matter of fact, the absence of all three would provide an eighth possibility.

Abramowitz (1937a) believed that all of the responses of the chromatophore systems which had been described might be explained in terms of one principle which he called ESH (eyestalk hormone). He believed that differences in the effectors together with differing threshold responses would account for the complicated responses of the chromatophore systems. This theory he called the "unitary theory" in contrast to the "multiple theory" proposed by Brown. However, even on the basis of his own theory, Abramowitz failed to explain satisfactorily the complex behavior found in *Palaemonetes* (Brown, 1934-1935a) or *Portunus* (Abramowitz, 1935). Despite this, Abramowitz continued to uphold the theory that a single hormone was present in the eyestalk, and subsequently set about assaying and attempting to purify it (Abramowitz, 1937b).

Hanström (1933, 1934) had earlier described two gland-like bodies in the eyestalk of crustaceans, the blood gland and the X-organ (Dohrn, 1906). In 1935, by dividing eyestalks of various crustaceans transversely into thirds, he found a correlation between that third possessing the blood gland (later called the sinus gland) and that possessing the chromatophorotropic activity. He concluded upon this circumstantial evidence that the sinus gland was the source of the principle. This conclusion was confirmed by Brown (1940) who made extracts of isolated sinus glands and compared their activity both quantitatively and qualitatively with extracts of both whole and sinus glandless stalks. The sinus gland appeared to be the only eyestalk gland concerned in the reactions examined.

A year following the discovery of the chromatophorotropic activity of the eyestalk, Koller (1930) brought forth evidence that the eyestalk hormone was concerned with calcium metabolism in *Crago*, since eyestalkless animals pos-

sessed less calcium in their molted exoskeletons than did normal ones.

Another function ascribed to an endocrine originating in the eyestalks is the control of retinal pigment migration. The retinal pigments are located within the ommatidial complex and are typically classifiable into three groups: (1) the proximal retinal pigment, (2) the distal retinal pigment, which are light-absorbing pigments, melanins, and (3) the white reflecting pigment, guanin. These three pigments assume different positions in the compound eye in light and in darkness. In many crustaceans these pigments continue to show rhythmical diurnal movements even when maintained under constant conditions of light. Kleinholz (1936) brought forth clear evidence for endocrine control of certain of these pigments. Injection of eyestalk extract into a dark-adapted *Palaemonetes* caused the distal retinal absorbing pigment and the white-reflecting pigment to assume the condition for light adaptation. The proximal retinal pigment, curiously enough, was unaffected. In 1938 Kleinholz concluded as did Hanström (1937b) that the hormone involved in this capacity must be different from those involved in integumentary pigment control since the retinal pigment activity was apparently quite independent of that of the integumentary chromatophores.

Welsh (1937) believed he had evidence that a principle originating in the eyestalk produced acceleration of heart rate in *Cambarus*, but during the following year performed other experiments which suggested that the effect was due to acetylcholine which could be extracted from crustacean nerve tissue quite generally. Scudamore (1941) however, presents evidence to prove that the sinus gland produces a principle which accelerates heart rate in the normal animal.

Another line of research has led to the conclusion that a hormone arising in the eyestalk is concerned with the control of molt and consequently is a factor influencing growth. Brown and Cunningham (1939) were able to accelerate molting rate by eyestalk extirpation and conversely were able to compensate partially for the effect of eyestalk removal by implanting sinus glands into the abdomens of the eyestalkless animals. Confirmation of molt acceleration following eyestalkectomy was ob-

tained by Smith (1940), Abramowitz and Abramowitz (1940), and by Kleinholz (1940). Abramowitz and Abramowitz also showed a growth rate greater than normal. Unfortunately none of these later workers attempted implantation of eyestalk tissue, or extract injections, to determine whether the effects were actually the result of a hormone deficiency.

Brown (1938), Brown and Cunningham (1939) and Smith (1940) reported greatly decreased viability of *Cambarus* following eyestalk removal. The reason for this effect is still far from clear.

Finally, of the many functions ascribed to an eyestalk hormone, we might mention the control of blood sugar concentration (Welsh, 1941). Injection of eyestalk extract results in a sudden modification in blood sugar.

With all of the more or less well demonstrated functions of hormones arising in the eyestalks of crustaceans it appears highly improbable that we are dealing here with a simple gland secreting a single principle. This is particularly evident when we recall, for instance, the demonstrated relative independence of the four pigments of *Palaemonetes* in normal color change, and the independent behavior of the retinal pigments with respect to the integumentary pigments. It appears far more likely that a number of hormones are present.

The first successful attempt to demonstrate more than one active fraction from sinus gland was made by Brown and Scudamore (1940). They found that when dried sinus glands were extracted with 100 per cent ethyl alcohol a fraction entered the alcohol which showed qualitatively different chromatophoretic properties from the fraction which was alcohol insoluble. Whereas extract of whole sinus gland strongly concentrated red pigment of *Palaemonetes* and strongly dispersed black pigment of *Uca*, the alcohol soluble fraction had almost no effect upon *Uca* black pigment while it had a strong effect upon *Palaemonetes* red. On the other hand, the alcohol insoluble fraction had a very strong dispersing action on *Uca* black but had a relatively weaker effect upon *Palaemonetes* red. Thus these results obviously could not be explained in terms of different concentrations of a single substance in the two fractions. A number of crabs and shrimp were examined and all

showed the presence of these two principles in varying proportions.

**Central Nervous Organs.**—It gradually became evident to certain of those working with crustacean chromatophores that the eyestalk was not the sole source of chromatophorotropic hormones. Eyestalkless animals showed chromatophore fluctuations and responses which could not be explained in other terms than variations in the concentrations of controlling hormones in the blood of these eyestalkless forms. Heterologous stimulation of the eyestubs of eyestalkless forms resulted in definite though transitory chromatophore responses by way of blood-borne agents. Brown (1933, 1935b) and Hosoi (1934) discovered that extracts of the nervous system of certain shrimp possessed chromatophorotropic activities. Kleinholz (1936) and Abramowitz (1937a) believed these effects were probably due to storage of an eyestalk principle in the central nervous organs. This explanation is quite untenable especially since the properties of this principle or complex of principles has been shown to be unlike eyestalk extract, even affecting certain chromatophores, the guanophores, in quite the reverse manner.

It appears highly likely that certain diurnal chromatophore changes which are known to occur in eyestalkless *Uca* and *Cambarus* result from a periodic liberation of such a principle from the nervous system.

**The Rostral Organ (Schwarz-Organ).**—Koller (1928), after confirming the discovery of Perkins that the eyestalks yielded an extract which would produce rapid blanching of the shrimp, reported that an antagonistic substance originated in the rostral region of *Crago*. Injection of extract of this region resulted in a darkening of the shrimps. The source of this hormone Koller named the "Schwarz-organ". With the exception of Beauvallet and Veil (1934) no one has been able to confirm the presence of such a rostral organ in shrimp, even in *Crago*. The matter appeared conclusively settled by the work of Kleinholz (1938) who carefully reinvestigated *Crago* obtained at the same place as Koller's animals. Kleinholz found no evidence for a schwarz-organ.

**Commissural Ganglia.**—The shrimp, *Crago*, possesses a very complex pig-

mentary system containing at least eight physiologically or morphologically different pigmentary types (Brown and Wulff, 1941). Despite the fact that this shrimp had been one of those most frequently investigated, yet there seemed to exist a very inadequate picture of the mechanism of pigmentary control. During the summer of 1939, Brown and Ederstrom (1940) reinvestigated this species. The investigation was commenced since the apparent final elimination of the "schwarz-organ" by Kleinholz left certain responses of eyestalkless animals (Brown, 1939) quite without explanation. If the "schwarz-organ" of Koller was non-existent then another source of a pigment dispersing agent seemed essential to explaining certain observations. Brown and Ederstrom noticed that within an hour after the removal of the eyestalks of *Crago* the telson and uropods become completely blanched and normally remain so. Stimulation of the eyestubs or other harsh stimulation resulted in a rapid darkening of the "tail"; this darkening persisted for one half to one hour and then disappeared. This was demonstrated to result from the activity of a blood-borne agent. A thorough survey of body tissues finally resulted in localizing the source of the hormone involved in the ganglia upon the circumoesophageal commissures. This principle was shown to be present in greater quantity in the commissures of white adapted animals than of black adapted ones. Thus it was demonstrated to be concerned with normal color changes in this animal.

Further investigation of the control of *Crago* chromatophores by Brown and Wulff (1941) led to the clear conclusion that in *Crago* the commissural ganglia are apparently as important in the hormonally controlled color changes of this form as are the much more publicized sinus glands. A number of pigments within the integumentary chromatophores are affected in opposite directions by extracts from the two sources. Other pigments are affected in the same direction by the two, but to differing degrees.

Evidence has been obtained demonstrating that the commissural ganglia contain more than a single chromatophorotropic principle. Ethyl alcohol extracts of the ganglia yield a fraction which is very active in blanching the



trunk of Crago. The "tail-darkening" principle of the ganglia is alcohol insoluble and remains in the residue after brief alcohol extraction. The alcohol soluble fraction appears at the present time to be similar to the principle which is found in all of the central nervous organs of Crustacea.

It is interesting, from the comparative standpoint, that the "Crago-tail-darkening principle" has been found in the commissural ganglia of the other shrimp but not of the crabs examined. Furthermore, a sinus gland principle which antagonizes the action of the commissural ganglia in tail darkening is found in the sinus glands of the shrimps but not of the crabs examined. This appears to be one of the first bits of evidence (see also Kleinholz, 1936) that the endocrine complex is not uniform throughout even the decapod crustaceans.

**The Gonads.**—Despite the fact that the earliest suspected endocrine activity within crustaceans was probably respecting a hormone influencing the development and maintenance of certain secondary sexual characteristics, yet this is still today not adequately established. The early suspicions were based upon many observations that when a male crab was castrated through parasitization by a rhizocephalan such as *Sacculina* there was a strong tendency for the animal to take on the external characteristics of the female sex. One of the more popular of the interpretations of this phenomenon was that the animal, as a result of the castration, was deprived of the source of a male sex hormone. These results may, however, be equally well interpreted in terms of modification of the general metabolism, or of specific metabolic processes by the activities of the parasite. These modifications might secondarily influence the secondary sexual characteristics.

Somewhat better evidence for the hormonal activities of crustacean gonads has come from such experiments as those of Haemmerli-Boveri (1926) working with *Asellus* and Callan (1940) working with *Leander*. These authors report that destruction of the ovaries by X-radiation results in failure of development in the case of certain female external characteristics concerned with incubation of the developing eggs.

The sum of all the innumerable reports on the subject of determination of

the secondary sex characteristics in Crustacea leaves one with the general impression that sex hormones are actually operative, but with little further idea as to the exact site of their formation or the extent of their action.

Definite demonstration of the presence of hormones concerned with the development of secondary and accessory sexual characteristics in crustaceans must be dependent upon the results of surgical removal of the hormone source and upon the results of effects of extracts or implants of this tissue source.

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